

10/697,036

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FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
LIFESCI' ENTERED AT 09:28:41 ON 18 JAN 2008

L1 38279 S BUDDING (W) YEAST?
L2 423082 S SACCHAROMYCES (W) CEREVISIAE
L3 430360 S L1 OR L2
L4 579 S (TRANSFORM? OR TRANSFECT?) (W) L3
L5 140 S HYBRID (W) SENSOR (W) KINASE?
L6 98 S OSMOSENSING (2W) KINASE?
L7 2 S L5 AND L6
L8 2 S L4 AND (L5 OR L6)
E NAKAJIMA H/AU
L9 9245 S E3
L10 1 S L4 AND L9
L11 10 S L5 AND L3
L12 4 DUP REM L11 (6 DUPLICATES REMOVED)

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NEWS	18	NOV 19	WPIX enhanced with XML display format
NEWS	19	NOV 30	ICSD reloaded with enhancements
NEWS	20	DEC 04	LINPADOCDB now available on STN
NEWS	21	DEC 14	BEILSTEIN pricing structure to change
NEWS	22	DEC 17	USPATOLD added to additional database clusters
NEWS	23	DEC 17	IMSDRUGCONF removed from database clusters and STN
NEWS	24	DEC 17	DGENE now includes more than 10 million sequences
NEWS	25	DEC 17	TOXCENTER enhanced with 2008 MeSH vocabulary in MEDLINE segment
NEWS	26	DEC 17	MEDLINE and LMEDLINE updated with 2008 MeSH vocabulary
NEWS	27	DEC 17	CA/Capplus enhanced with new custom IPC display formats
NEWS	28	DEC 17	STN Viewer enhanced with full-text patent content from USPATOLD
NEWS	29	JAN 02	STN pricing information for 2008 now available
NEWS	30	JAN 16	CAS patent coverage enhanced to include exemplified prophetic substances

NEWS EXPRESS 19 SEPTEMBER 2007: CURRENT WINDOWS VERSION IS V8.2, CURRENT MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP), AND CURRENT DISCOVER FILE IS DATED 19 SEPTEMBER 2007.

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FILE 'LIFESCI' ENTERED AT 09:28:41 ON 18 JAN 2008
COPYRIGHT (C) 2008 Cambridge Scientific Abstracts (CSA)

=> s budding (w) yeast?
L1 38279 BUDDING (W) YEAST?

=> s saccharomyces (w)cerevisiae
L2 423082 SACCHAROMYCES (W) CEREVISIAE

=> s l1 or l2
L3 430360 L1 OR L2

=> s (transform? or transfect?)(w)l3
L4 579 (TRANSFORM? OR TRANSFECT?)(W) L3

=> s hybrid (w)sensor(w)kinase?
L5 140 HYBRID (W) SENSOR(W) KINASE?

=> s osmosensing (2w)kinase?
L6 98 OSMOSENSING (2W) KINASE?

=> s 15 and 16

L7 2 L5 AND L6

=> d 1-2 ibib ab

L7 ANSWER 1 OF 2 BIOTECHDS COPYRIGHT 2008 THE THOMSON CORP. on STN

ACCESSION NUMBER: 2004-15129 BIOTECHDS

TITLE: New transformed cell in which a polynucleotide coding for osmosensing histidine kinase having no transmembrane region has been introduced, useful for identifying an antifungal compound useful for killing a fungus;
vector expression in host cell for use in drug screening and fungus infection therapy

AUTHOR: NAKAJIMA H

PATENT ASSIGNEE: SUMITOMO CHEM CO LTD

PATENT INFO: EP 1415996 6 May 2004

APPLICATION INFO: EP 2003-256895 30 Oct 2003

PRIORITY INFO: JP 2002-317736 31 Oct 2002; JP 2002-317736 31 Oct 2002

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2004-341880 [32]

AB DERWENT ABSTRACT:

NOVELTY - A transformed cell in which a polynucleotide having a sequence encoding an amino acid sequence of an osmosensing histidine kinase having no transmembrane region has been introduced in a functional form into a cell deficient in at least one hybrid-sensor kinase, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) assaying the antifungal activity of a substance; (2) identifying an antifungal compound; (3) an antifungal compound selected by the method above; (4) killing a fungus; (5) an osmosensing histidine kinase having no transmembrane region and derived from a plant-pathogenic filamentous fungus or which has an amino acid sequence selected from: (a) a sequence of 1293, 1307 or 1438 amino acids (SEQ ID NO: 41, 55 or 68) given in the specification or a sequence 95% or more homologous to SEQ ID NO: 41, 55 or 68; (b) a sequence encoded by a DNA amplified by PCR using a *Fusarium oxysporum*-derived cDNA as a template and using oligonucleotides having the nucleotide sequences tgcaactagtagtgggtgacgacgacgagccctcgc (SEQ ID NO: 52) and gagctgcagtttagttggaagacttcgcataatc (SEQ ID NO: 53) as primers; (c) a sequence encoded by a DNA amplified by PCR using *Mycosphaerella tritici*-derived cDNA as a template and using oligonucleotides having the sequences ccactagtagtgcgtgcaagaagagacttcg (SEQ ID NO: 64) and cctaagcttctcagctgctatgggcacgaa (SEQ ID NO: 65) as primers; (d) a sequence encoded by a DNA amplified by PCR using *Thanapethorus cucumeris*-derived cDNA as a template and using oligonucleotides having the sequences ggaactagtagtggcaggtacaacggggggacacc (SEQ ID NO: 85) and tgcaagcttttagtgggcaccgtggggtgttacg (SEQ ID NO: 86) as primers; and (e) a sequence derived from *Phytophthora infestans* and has the amino acid sequence of 124 amino acids (SEQ ID NO: 90) given in the specification; (6) a polynucleotide having a nucleotide sequence encoding an amino acid sequence of an osmosensing histidine kinase having no transmembrane region derived from a plant-pathogen filamentous fungus described above or having a sequence of 3882, 3924, or 4317 bp (SEQ ID NO: 42, 56, or 69) given in the specification; (7) obtaining the polynucleotide above; and (8) an oligonucleotide which comprises a nucleotide sequence selected from 17 sequences of 23-34 bp (SEQ ID NO: 30-40, 52, 53, 64, 65, 85, and 86) given in the specification, e.g., aacatgtcccacgarattcgmacacc (SEQ ID NO: 30) caccgagattcgvacacccatgaaygg (SEQ ID NO: 31) aggccttccaaaaggctctvcggga (SEQ ID NO: 32) gagatggaccctgaaatcacmac (SEQ ID NO: 33) cagatattctcyagyaagtytckcg (SEQ ID NO: 34) atagcrttgccaacmaggttmagaataa (SEQ ID NO: 35)

aacttgatggcrttkccaacmaggtt (SEQ ID NO: 36) ctctgtgaacttgatrgcrttkccaac
(SEQ ID NO: 37) atacacttttcncgggtcacccatcat (SEQ ID NO: 38)
tccatctgbgccttgatacacttttc (SEQ ID NO: 39) ggcttygavagatactcgtccatctg
(SEQ ID NO: 40).

BIOTECHNOLOGY - Preferred Transformed Cell: The polynucleotide is a polynucleotide complementing the deficiency in hybrid-sensor kinase in the cell deficient in at least one hybrid-sensor kinase in which the polynucleotide has been introduced. The cell is a microorganism, particularly budding yeast. The osmosensing histidine kinase having no transmembrane region is an osmosensing histidine kinase having no transmembrane region and having a mutation which confers resistance to any of a dicarboxylimide antifungal compound, an aromatic hydrocarbon antifungal compound and a phenylpyrrole antifungal compound to the cell. The osmosensing histidine kinase is derived from a plant-pathogenic filamentous fungus and has no transmembrane region. The osmosensing histidine kinase has an amino acid sequence of 1315, 1307, 1293, 1307, 1438 or 124 amino acids (SEQ ID NO: 1, 16, 41, 55, 68 or 90, respectively) given in the specification. The nucleotide sequence encoding an amino acid sequence of the osmosensing histidine kinase is a sequence of 3948, 3924, 3882, 3924, or 4317 bp (SEQ ID NO: 2, 17, 42, 56, or 69, respectively) also given in the specification. **Preferred Method:** Assaying the antifungal activity of a substance comprises culturing a transformed cell defined above in the presence of a test substance, measuring an amount of intracellular signal transduction from the osmosensing histidine kinase having no transmembrane region expressed in the cultured transformed cell or an index value having the correlation, and assessing the antifungal activity of the test substance based on a difference between an amount of intracellular signal transduction or an index value having the correlation measured and a control. The amount of intracellular signal transduction or the index value having the correlation is an amount of growth of the transformed cell. Identifying an antifungal compound comprises selecting an antifungal compound based on the antifungal activity assessed in the assaying method defined above. Killing a fungus comprises identifying an antifungal compound by the method above and contacting the fungus with the identified antifungal compound. Obtaining the polynucleotide above comprises amplifying a desired polynucleotide by PCR using the oligonucleotide above and recovering the amplified desired polynucleotide.

ACTIVITY - Fungicide. No biological data given.

MECHANISM OF ACTION - None given.

USE - The transformed cell is useful for assaying the antifungal activity of a substance and identifying an antifungal compound which is useful for killing a fungus (claimed).

EXAMPLE - BcOS-1 DNA was cloned into a shuttle vector p415ADH (ATCC87312) replicable in yeast and *Escherichia coli*. Both were digested, separated by agarose gel electrophoresis, and a part of the gel containing a desired DNA was excised. The BcOS-1 DNA was inserted between *Spe*I and *Pst*I sites in the multicloning site of the shuttle vector. A nucleotide sequence of the resulting expression plasmid was analyzed after a sequencing reaction. The nucleotide sequence of 3948 bp (SEQ ID NO: 2) given in the specification was obtained and it was confirmed that the expression plasmid pADHBcOS1 harbored a DNA having a nucleotide sequence encoding an amino acid sequence of BcOS-1. The prepared expression plasmid was introduced into each of budding yeast (*Saccharomyces cerevisiae* AH22 strain) (IFO10144) and TM182 strain. By utilizing disappearance of leucine auxotrophy in the resulting transformed budding yeast, the transformed budding yeast AH22 strain was selected on a Glu-Leu agar medium, and the transformed budding yeast TM182 was selected on a Gal-Ura-Leu agar medium. It was confirmed that the resulting TM182-BcOS1 grows even when transplanted to a Glu-Ura-Leu medium. (211 pages)

L7 ANSWER 2 OF 2 HCAPLUS COPYRIGHT 2008 ACS on STN

ACCESSION NUMBER: 2004:370684 HCAPLUS

DOCUMENT NUMBER: 140:369919

TITLE: Transformed cell with enhanced sensitivity to antifungal compound, expressing mutated gene, os-1, for an osmosensing histidine kinase, and uses for fungicide screening

INVENTOR(S): Nakajima, Hiroki

PATENT ASSIGNEE(S): Sumitomo Chemical Company, Limited, Japan

SOURCE: Eur. Pat. Appl., 211 pp.

CODEN: EPXXDW

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 1415996	A2	20040506	EP 2003-256895	20031030
EP 1415996	A3	20040901		
EP 1415996	B1	20071017		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK				
JP 2005087182	A	20050407	JP 2003-354761	20031015
SG 127705	A1	20061229	SG 2003-6525	20031030
AT 375997	T	20071115	AT 2003-256895	20031030
US 2004137594	A1	20040715	US 2003-697036	20031031
PRIORITY APPLN. INFO.:			JP 2002-317736	A 20021031
			JP 2003-207458	A 20030813

AB An object of the present invention is to provide a method of detecting the antifungal activity and a method of antifungal screening using filamentous fungi homologs of *Neurispora crassa* os-1 gene encoding a two-component system osmosensing histidine kinase having no transmembrane region. OS-1 protein and cDNA sequences from phytopathogenic fungi, including *Botryotinia fuckeliana* (BcOS-1), *Magnaiorthe grisea* (HIK1), *Fusarium oxysporum* (FoOS-1), *Mycosphaerella tritici* (StOS-1), *Thanatephorus cucumeris* (RsOS-1), and *Phytophthora infestans* (PiOS-1), are provided. The present invention provides transformed cells (such as budding yeast) in which a os-1 gene homolog encoding an osmosensing histidine kinase having no transmembrane region has been introduced in a functional form into a cell deficient in at least one hybrid-sensor kinase. The os-1 transgene is carrying a mutation which confers resistance to the cell to any of a dicarboxyimide antifungal compound, an aromatic hydrocarbon antifungal compound and a phenylpyrrole antifungal compound. Provided are a method of assaying the antifungal activity of a test substance using the transformed cell, and a method of identifying an antifungal compound

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=> s 14 and (15 or 16)
L8 2 L4 AND (L5 OR L6)

=> d 1-2 ibib ab

L8 ANSWER 1 OF 2 BIOTECHDS COPYRIGHT 2008 THE THOMSON CORP. on STN

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PATENT ASSIGNEE: SUMITOMO CHEM CO LTD

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aacttgatggcrttkccaacmaggtt (SEQ ID NO: 36) ctctgtgaacttgatrgcrttkccaac
(SEQ ID NO: 37) atacacttttcncgggtcacccatcat (SEQ ID NO: 38)
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L8 ANSWER 2 OF 2 HCAPLUS COPYRIGHT 2008 ACS on STN

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DOCUMENT NUMBER: 140:369919

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INVENTOR(S): Nakajima, Hiroki

PATENT ASSIGNEE(S): Sumitomo Chemical Company, Limited, Japan

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JP 2005087182	A	20050407	JP 2003-354761	20031015
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The os-1 transgene is carrying a mutation which confers resistance to the cell to any of a dicarboxyimide antifungal compound, an aromatic hydrocarbon antifungal compound and a phenylpyrrole antifungal compound. Provided are a method of assaying the antifungal activity of a test substance using the transformed cell, and a method of identifying an antifungal compound

=> e nakajima h/au

E1	5	NAKAJIMA GORO/AU
E2	1	NAKAJIMA GOZO/AU
E3	9245 -->	NAKAJIMA H/AU
E4	4	NAKAJIMA H */AU
E5	19	NAKAJIMA H H/AU
E6	56	NAKAJIMA H O/AU
E7	14	NAKAJIMA HACHIRO/AU
E8	14	NAKAJIMA HADJIME/AU
E9	152	NAKAJIMA HAJIME/AU
E10	1	NAKAJIMA HANAE/AU
E11	5	NAKAJIMA HANAKO/AU

E12 8 NAKAJIMA HARU/AU

=> s e3

L9 9245 "NAKAJIMA H"/AU

=> d his

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=> s l4 and l9

L10 1 L4 AND L9

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L10 ANSWER 1 OF 1 BIOTECHDS COPYRIGHT 2008 THE THOMSON CORP. on STN

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AB DERWENT ABSTRACT:

NOVELTY - A transformed cell in which a polynucleotide having a sequence encoding an amino acid sequence of an osmosensing histidine kinase having no transmembrane region has been introduced in a functional form into a cell deficient in at least one hybrid-sensor kinase, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) assaying the antifungal activity of a substance; (2) identifying an antifungal compound; (3) an antifungal compound selected by the method above; (4) killing a fungus; (5) an osmosensing histidine kinase having no transmembrane region and derived from a plant-pathogenic filamentous fungus or which has an amino acid sequence selected from: (a) a sequence of 1293, 1307 or 1438 amino acids (SEQ ID NO: 41, 55 or 68) given in the specification or a sequence 95% or more homologous to SEQ ID NO: 41, 55 or 68; (b) a sequence encoded by a DNA amplified by PCR using a *Fusarium oxysporum*-derived cDNA as a template and using oligonucleotides having the nucleotide sequences *tgactagtagtggttgacgacgcggccctcgc* (SEQ ID NO: 52) and *gagctgcagtttagttggaagacttcgcata* (SEQ ID NO: 53) as primers; (c) a sequence encoded by a DNA amplified by PCR using *Mycosphaerella tritici*-derived cDNA as a template and using oligonucleotides having the sequences *cccactagtagtgctgcaagaagagacttcg* (SEQ ID NO: 64) and *cctaagcttctcagctgctatgggcacgaa* (SEQ ID NO: 65) as primers; (d) a sequence

encoded by a DNA amplified by PCR using *Thanapethorus cucumeris*-derived cDNA as a template and using oligonucleotides having the sequences ggaactagtagtggcaggtacaacggggggacacc (SEQ ID NO: 85) and tgcaagcttttagtgggcaccgtggggtgttacg (SEQ ID NO: 86) as primers; and (e) a sequence derived from *Phytophthora infestans* and has the amino acid sequence of 124 amino acids (SEQ ID NO: 90) given in the specification; (6) a polynucleotide having a nucleotide sequence encoding an amino acid sequence of an osmosensing histidine kinase having no transmembrane region derived from a plant-pathogen filamentous fungus described above or having a sequence of 3882, 3924, or 4317 bp (SEQ ID NO: 42, 56, or 69) given in the specification; (7) obtaining the polynucleotide above; and (8) an oligonucleotide which comprises a nucleotide sequence selected from 17 sequences of 23-34 bp (SEQ ID NO: 30-40, 52, 53, 64, 65, 85, and 86) given in the specification, e.g., aacatgtcccacgarattcgmacacc (SEQ ID NO: 30) caccgagattcgvacacccatgaaygg (SEQ ID NO: 31) aggccttccaaaaggctctvcggga (SEQ ID NO: 32) gagatggaccctgaaatcacmac (SEQ ID NO: 33) cagatattctcyagygaagtytckcg (SEQ ID NO: 34) atagcrttgccaacmaggttmagaataa (SEQ ID NO: 35) aacttgatggcrttkccaacmaggtt (SEQ ID NO: 36) ctctgtgaacttgatrgcrttkccaac (SEQ ID NO: 37) atacacttttncnggtcacccatcat (SEQ ID NO: 38) tccatctgbgcttgatacacttttc (SEQ ID NO: 39) ggcttvagavatactcgtccatctg (SEQ ID NO: 40).

BIOTECHNOLOGY - Preferred Transformed Cell: The polynucleotide is a polynucleotide complementing the deficiency in hybrid-sensor kinase in the cell deficient in at least one hybrid-sensor kinase in which the polynucleotide has been introduced. The cell is a microorganism, particularly budding yeast. The osmosensing histidine kinase having no transmembrane region is an osmosensing histidine kinase having no transmembrane region and having a mutation which confers resistance to any of a dicarboxylimide antifungal compound, an aromatic hydrocarbon antifungal compound and a phenylpyrrole antifungal compound to the cell. The osmosensing histidine kinase is derived from a plant-pathogenic filamentous fungus and has no transmembrane region. The osmosensing histidine kinase has an amino acid sequence of 1315, 1307, 1293, 1307, 1438 or 124 amino acids (SEQ ID NO: 1, 16, 41, 55, 68 or 90, respectively) given in the specification. The nucleotide sequence encoding an amino acid sequence of the osmosensing histidine kinase is a sequence of 3948, 3924, 3882, 3924, or 4317 bp (SEQ ID NO: 2, 17, 42, 56, or 69, respectively) also given in the specification. **Preferred Method:** Assaying the antifungal activity of a substance comprises culturing a transformed cell defined above in the presence of a test substance, measuring an amount of intracellular signal transduction from the osmosensing histidine kinase having no transmembrane region expressed in the cultured transformed cell or an index value having the correlation, and assessing the antifungal activity of the test substance based on a difference between an amount of intracellular signal transduction or an index value having the correlation measured and a control. The amount of intracellular signal transduction or the index value having the correlation is an amount of growth of the transformed cell. Identifying an antifungal compound comprises selecting an antifungal compound based on the antifungal activity assessed in the assaying method defined above. Killing a fungus comprises identifying an antifungal compound by the method above and contacting the fungus with the identified antifungal compound. Obtaining the polynucleotide above comprises amplifying a desired polynucleotide by PCR using the oligonucleotide above and recovering the amplified desired polynucleotide.

ACTIVITY - Fungicide. No biological data given.

MECHANISM OF ACTION - None given.

USE - The transformed cell is useful for assaying the antifungal activity of a substance and identifying an antifungal compound which is useful for killing a fungus (claimed).

EXAMPLE - BcOS-1 DNA was cloned into a shuttle vector p415ADH (ATCC87312) replicable in yeast and *Escherichia coli*. Both were digested, separated by agarose gel electrophoresis, and a part of the gel containing a desired DNA was excised. The BcOS-1 DNA was inserted between

SpeI and PstI sites in the multicloning site of the shuttle vector. A nucleotide sequence of the resulting expression plasmid was analyzed after a sequencing reaction. The nucleotide sequence of 3948 bp (SEQ ID NO: 2) given in the specification was obtained and it was confirmed that the expression plasmid pADHBcOS1 harbored a DNA having a nucleotide sequence encoding an amino acid sequence of BcOS-1. The prepared expression plasmid was introduced into each of budding yeast (*Saccharomyces cerevisiae* AH22 strain) (IFO10144) and TM182 strain. By utilizing disappearance of leucine auxotrophy in the resulting transformed budding yeast, the transformed budding yeast AH22 strain was selected on a Glu-Leu agar medium, and the transformed budding yeast TM182 was selected on a Gal-Ura-Leu agar medium. It was confirmed that the resulting TM182-BcOS1 grows even when transplanted to a Glu-Ura-Leu medium. (211 pages)

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(FILE 'HOME' ENTERED AT 09:28:11 ON 18 JAN 2008)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 09:28:41 ON 18 JAN 2008

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L1      38279 S BUDDING (W) YEAST?
L2      423082 S SACCHAROMYCES (W)CEREVISIAE
L3      430360 S L1 OR L2
L4      579 S (TRANSFORM? OR TRANSFECT?)(W)L3
L5      140 S HYBRID (W)SENSOR(W)KINASE?
L6      98 S OSMOSENSING (2W)KINASE?
L7      2 S L5 AND L6
L8      2 S L4 AND (L5 OR L6)
        E NAKAJIMA H/AU
L9      9245 S E3
L10     1 S L4 AND L9
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L12 ANSWER 1 OF 4 HCAPLUS COPYRIGHT 2008 ACS on STN

ACCESSION NUMBER: 2006:1145495 HCAPLUS

TITLE: Signal transduction in yeast involving a His-to-Asp phosphorelay system

AUTHOR(S): West, Ann H.; Xu, Qingping; Porter, Stace; Janiak-Spens, Fabiola; Chooback, Lilian

CORPORATE SOURCE: Department of Chemistry and Biochemistry, University of Oklahoma, Norman, OK, 73019, USA

SOURCE: Abstracts, 62nd Southwest Regional Meeting of the American Chemical Society, Houston, TX, United States, October 19-22 (2006), SRM-131. American Chemical Society: Washington, D. C.

CODEN: 69INSW

DOCUMENT TYPE: Conference; Meeting Abstract

LANGUAGE: English

AB Histidine-containing phosphotransfer (HPT) proteins play an essential role in the transfer of phosphoryl groups between response regulator domains in multi-step His-Asp signal transduction systems. In *Saccharomyces cerevisiae*, the HPT protein YPD1 facilitates phosphoryl group transfer from the response regulator domain of a membrane-bound hybrid sensor kinase (SLN1) to two downstream response regulators SSK1 and SKN7, which are involved in hyperosmotic and oxidative stress responses, resp. Protein-protein interactions involving signaling partners are transient and phosphorylation-dependent. This talk will focus on the mol. basis of interaction of YPD1 with each of the three response regulator domains (SLN1-R1, SSK1-R2, and SKN7-R3, resp.), which we have investigated through a variety of means, including a comparative yeast two-hybrid interaction assay, X-ray crystallog. anal. of a YPD1/SLN1-R1 complex, and kinetic anal. of YPD1-dependent phosphotransfer reactions.

L12 ANSWER 2 OF 4 BIOTECHDS COPYRIGHT 2008 THE THOMSON CORP. on STN

ACCESSION NUMBER: 2005-14092 BIOTECHDS

TITLE: Novel transformed cell produced by transducing gene encoding osmotic-pressure sensitive histidine kinase that functions on cell lacking hybrid sensor kinase function, useful for screening antimicrobial agents; yeast host cell transformation using fungus enzyme gene for use in antimicrobial substance screening

PATENT ASSIGNEE: SUMITOMO CHEM CO LTD

PATENT INFO: JP 2005087182 7 Apr 2005

APPLICATION INFO: JP 2003-354761 15 Oct 2003

PRIORITY INFO: JP 2003-207458 13 Aug 2003; JP 2002-317736 31 Oct 2002

DOCUMENT TYPE: Patent

LANGUAGE: Japanese

OTHER SOURCE: WPI: 2005-288601 [30]

AB DERWENT ABSTRACT:

NOVELTY - A transformed cell (I), produced by transducing a gene having a base sequence encoding osmotic-pressure sensitive histidine kinase that does not have cytoplasmic membrane penetration region that functions on the cell, where the cell lacks the hybrid sensor kinase function, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following: (1) testing (M1) antimicrobial activity of a substance; (2) screening (M2) anti-microbial substance; (3) anti-microbial substance (II) screened by (M2); (4) pathogenic, filamentous plant fungi (III), comprising osmotic pressure sensitive histidine kinase that does not have cytoplasmic membrane penetration region, or a polynucleotide encoding osmotic pressure sensitive histidine kinase lacking cytoplasmic membrane penetration region; (5) osmotic pressure sensitive histidine kinase lacking cytoplasmic membrane penetration region (IV), comprising a fully defined 1293 amino acid (SEQ ID No:41), 1307 amino acid (SEQ ID No:55), 1438 amino acid (SEQ ID No:68) sequences given in the specification, one

or more addition, deletion or substitution in SEQ ID No:41, 55 and 69, and SEQ ID No:41 and SEQ ID No: 55 having 95% or more sequence identity with SEQ ID No:68, or a base sequence encoding SEQ ID No:41, 55 and 68; (6) polynucleotide (V) having a fully defined 3882 nucleotide (SEQ ID No:42), 3924 nucleotide (SEQ ID No:56) and 4317 nucleotide (SEQ ID No:69) sequences given in the specification; (7) acquisition method of a polynucleotide encoding osmotic pressure sensitive histidine kinase lacking cytoplasmic membrane penetration region; and (8) oligonucleotide comprising a fully defined 10 nucleotide sequence having approximately 23-28 nucleotide (SEQ ID No:30-40) sequences given in the specification, a fully defined 32 nucleotide (SEQ ID No:52), 33 nucleotide (SEQ ID No:53), 30 nucleotide (SEQ ID No:64), 30 nucleotide (SEQ ID No:65), 34 nucleotide (SEQ ID No:85) and 34 nucleotide (SEQ ID No:86) sequences given in the specification.

BIOTECHNOLOGY - Preferred Method: In (I), the gene encoding osmotic-pressure sensitive histidine kinase lacking cytoplasmic membrane penetration region, complements the function of deleted hybrid sensor kinase within the cell lacking hybrid sensor kinase. The transformed cell is a microorganism, preferably budding yeast. The osmotic-pressure sensitive histidine kinase has resistance with respect to dicarboxyimide, aromatic-hydrocarbon or phenyl-pyrrole antimicrobial substance. The osmotic-pressure sensitive histidine kinase is of plant pathogenic mold origin, preferably botrytis disease filamentous fungi, rice-blight filamentous fungi, spinach wilt-disease filamentous fungi, wheat leaf-blight filamentous fungi, rice sheath-blight-disease filament form, or tomato late blight filamentous-fungi origin, and lacks cytoplasmic-membrane penetration region. The osmotic-pressure sensitive histidine kinase has a fully defined 1315 amino acid (SEQ ID No:1), 1307 amino acid (SEQ ID No:16), 1293 amino acid (SEQ ID No:41), 1307 amino acids (SEQ ID No:55) and 1438 amino acids (SEQ ID No:68) sequences given in the specification. The base sequence encoding osmotic-pressure sensitive histidine kinase, has a fully defined 3948 nucleotide (SEQ ID No:2), 3924 nucleotide (SEQ ID No:17), 3882 nucleotide (SEQ ID No:42), 3924 nucleotide (SEQ ID No:56), 4317 nucleotide (SEQ ID No:69) sequences given in the specification.

ACTIVITY - Antimicrobial. No biological data is given.

MECHANISM OF ACTION - Osmotic-pressure sensitive histidine kinase inhibitor.

USE - (I) is useful for screening antimicrobial substances (claimed).

ADVANTAGE - The sensitivity to antimicrobial substance, is increased. (54 pages)

L12 ANSWER 3 OF 4 BIOTECHDS COPYRIGHT 2008 THE THOMSON CORP. on STN
DUPLICATE 1

ACCESSION NUMBER: 2004-15129 BIOTECHDS

TITLE: New transformed cell in which a polynucleotide coding for osmosensing histidine kinase having no transmembrane region has been introduced, useful for identifying an antifungal compound useful for killing a fungus;
vector expression in host cell for use in drug screening and fungus infection therapy

AUTHOR: NAKAJIMA H

PATENT ASSIGNEE: SUMITOMO CHEM CO LTD

PATENT INFO: EP 1415996 6 May 2004

APPLICATION INFO: EP 2003-256895 30 Oct 2003

PRIORITY INFO: JP 2002-317736 31 Oct 2002; JP 2002-317736 31 Oct 2002

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2004-341880 [32]

AB DERWENT ABSTRACT:

NOVELTY - A transformed cell in which a polynucleotide having a sequence encoding an amino acid sequence of an osmosensing histidine kinase having

no transmembrane region has been introduced in a functional form into a cell deficient in at least one hybrid-sensor kinase, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) assaying the antifungal activity of a substance; (2) identifying an antifungal compound; (3) an antifungal compound selected by the method above; (4) killing a fungus; (5) an osmosensing histidine kinase having no transmembrane region and derived from a plant-pathogenic filamentous fungus or which has an amino acid sequence selected from: (a) a sequence of 1293, 1307 or 1438 amino acids (SEQ ID NO: 41, 55 or 68) given in the specification or a sequence 95% or more homologous to SEQ ID NO: 41, 55 or 68; (b) a sequence encoded by a DNA amplified by PCR using a *Fusarium oxysporum*-derived cDNA as a template and using oligonucleotides having the nucleotide sequences *tgcaactagtagtggtgacgacgcggccctcgc* (SEQ ID NO: 52) and *gagctgcagtttagttggaagacttcgcatatc* (SEQ ID NO: 53) as primers; (c) a sequence encoded by a DNA amplified by PCR using *Mycosphaerella tritici*-derived cDNA as a template and using oligonucleotides having the sequences *cccactagtagtgctgcaagaagagacttcg* (SEQ ID NO: 64) and *cctaagcttctcagctgctatgggcacgaa* (SEQ ID NO: 65) as primers; (d) a sequence encoded by a DNA amplified by PCR using *Thanapethorus cucumeris*-derived cDNA as a template and using oligonucleotides having the sequences *ggaactagtagtgacaggtacaacggggggacacc* (SEQ ID NO: 85) and *tgcaagcttttagtgggcaccgtgggtgttacg* (SEQ ID NO: 86) as primers; and (e) a sequence derived from *Phytophthora infestans* and has the amino acid sequence of 124 amino acids (SEQ ID NO: 90) given in the specification; (6) a polynucleotide having a nucleotide sequence encoding an amino acid sequence of an osmosensing histidine kinase having no transmembrane region derived from a plant-pathogen filamentous fungus described above or having a sequence of 3882, 3924, or 4317 bp (SEQ ID NO: 42, 56, or 69) given in the specification; (7) obtaining the polynucleotide above; and (8) an oligonucleotide which comprises a nucleotide sequence selected from 17 sequences of 23-34 bp (SEQ ID NO: 30-40, 52, 53, 64, 65, 85, and 86) given in the specification, e.g., *aacatgtcccacgarattcgmacacc* (SEQ ID NO: 30) *caccgagattcgvacacccatgaaygg* (SEQ ID NO: 31) *aggccttccaaaaggctctvcggga* (SEQ ID NO: 32) *gagatggaccctgaaatcacmac* (SEQ ID NO: 33) *cgatatattctcyaggyaagtytckcg* (SEQ ID NO: 34) *atagcrttgccaacmaggttmagaataa* (SEQ ID NO: 35) *aacttgatggcrttkccaacmaggtt* (SEQ ID NO: 36) *ctctgtgaacttgatrgcrttkccaac* (SEQ ID NO: 37) *atacacttttncgggtcacccatcat* (SEQ ID NO: 38) *tccatctgbgcctggatacacttttc* (SEQ ID NO: 39) *ggcttvagavagatactgcctcatctg* (SEQ ID NO: 40).

BIOTECHNOLOGY - Preferred Transformed Cell: The polynucleotide is a polynucleotide complementing the deficiency in hybrid-sensor kinase in the cell deficient in at least one hybrid-sensor kinase in which the polynucleotide has been introduced. The cell is a microorganism, particularly budding yeast. The osmosensing histidine kinase having no transmembrane region is an osmosensing histidine kinase having no transmembrane region and having a mutation which confers resistance to any of a dicarboxylimide antifungal compound, an aromatic hydrocarbon antifungal compound and a phenylpyrrole antifungal compound to the cell. The osmosensing histidine kinase is derived from a plant-pathogenic filamentous fungus and has no transmembrane region. The osmosensing histidine kinase has an amino acid sequence of 1315, 1307, 1293, 1307, 1438 or 124 amino acids (SEQ ID NO: 1, 16, 41, 55, 68 or 90, respectively) given in the specification. The nucleotide sequence encoding an amino acid sequence of the osmosensing histidine kinase is a sequence of 3948, 3924, 3882, 3924, or 4317 bp (SEQ ID NO: 2, 17, 42, 56, or 69, respectively) also given in the specification. Preferred Method: Assaying the antifungal activity of a substance comprises culturing a transformed cell defined above in the presence of a test substance, measuring an amount of intracellular signal transduction from the osmosensing histidine kinase having no transmembrane region expressed in the cultured transformed cell or an index value having the correlation, and assessing the antifungal activity of the test substance based on a

difference between an amount of intracellular signal transduction or an index value having the correlation measured and a control. The amount of intracellular signal transduction or the index value having the correlation is an amount of growth of the transformed cell. Identifying an antifungal compound comprises selecting an antifungal compound based on the antifungal activity assessed in the assaying method defined above. Killing a fungus comprises identifying an antifungal compound by the method above and contacting the fungus with the identified antifungal compound. Obtaining the polynucleotide above comprises amplifying a desired polynucleotide by PCR using the oligonucleotide above and recovering the amplified desired polynucleotide.

ACTIVITY - Fungicide. No biological data given.

MECHANISM OF ACTION - None given.

USE - The transformed cell is useful for assaying the antifungal activity of a substance and identifying an antifungal compound which is useful for killing a fungus (claimed).

EXAMPLE - BcOS-1 DNA was cloned into a shuttle vector p415ADH (ATCC87312) replicable in yeast and Escherichia coli. Both were digested, separated by agarose gel electrophoresis, and a part of the gel containing a desired DNA was excised. The BcOS-1 DNA was inserted between SpeI and PstI sites in the multicloning site of the shuttle vector. A nucleotide sequence of the resulting expression plasmid was analyzed after a sequencing reaction. The nucleotide sequence of 3948 bp (SEQ ID NO: 2) given in the specification was obtained and it was confirmed that the expression plasmid pADHBcOS1 harbored a DNA having a nucleotide sequence encoding an amino acid sequence of BcOS-1. The prepared expression plasmid was introduced into each of budding yeast (*Saccharomyces cerevisiae* AH22 strain) (IFO10144) and TM182 strain. By utilizing disappearance of leucine auxotrophy in the resulting transformed budding yeast, the transformed budding yeast AH22 strain was selected on a Glu-Leu agar medium, and the transformed budding yeast TM182 was selected on a Gal-Ura-Leu agar medium. It was confirmed that the resulting TM182-BcOS1 grows even when transplanted to a Glu-Ura-Leu medium. (211 pages)

L12 ANSWER 4 OF 4 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 2001076412 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11073911
TITLE: Novel role for an HPT domain in stabilizing the phosphorylated state of a response regulator domain.
AUTHOR: Janiak-Spens F; Sparling D P; West A H
CORPORATE SOURCE: Department of Chemistry and Biochemistry, University of Oklahoma, Norman, Oklahoma 73019, USA.
CONTRACT NUMBER: GM59311 (NIGMS)
SOURCE: Journal of bacteriology, (2000 Dec) Vol. 182, No. 23, pp. 6673-8.
Journal code: 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, U.S. GOV'T, NON-P.H.S.)
(RESEARCH SUPPORT, U.S. GOV'T, P.H.S.)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200101
ENTRY DATE: Entered STN: 22 Mar 2001
Last Updated on STN: 14 Feb 2003
Entered Medline: 11 Jan 2001
AB Two-component regulatory systems that utilize a multistep phosphorelay mechanism often involve a histidine-containing phosphotransfer (HPT) domain. These HPT domains serve an essential role as histidine-phosphorylated protein intermediates during phosphoryl transfer from one response regulator domain to another. In *Saccharomyces cerevisiae*, the YPD1 protein facilitates phosphoryl transfer from

a hybrid sensor kinase, SLN1, to two distinct response regulator proteins, SSK1 and SKN7. Because the phosphorylation state largely determines the functional state of response regulator proteins, we have carried out a comparative study of the phosphorylated lifetimes of the three response regulator domains associated with SLN1, SSK1, and SKN7 (R1, R2, and R3, respectively). The isolated regulatory domains exhibited phosphorylated lifetimes within the range previously observed for other response regulator domains (i.e., several minutes to several hours). However, in the presence of YPD1, we found that the half-life of phosphorylated SSK1-R2 was dramatically extended (almost 200-fold longer than in the absence of YPD1). This stabilization effect was specific for SSK1-R2 and was not observed for SLN1-R1 or SKN7-R3. Our findings suggest a mechanism by which SSK1 is maintained in its phosphorylated state under normal physiological conditions and demonstrate an unprecedented regulatory role for an HPT domain in a phosphorelay signaling system.

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EAST Search History

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	1230	budding adj yeast\$2	US-PGPUB; USPAT	OR	OFF	2008/01/18 09:36
L2	30715	saccharomyces adj cerevisiae	US-PGPUB; USPAT	OR	OFF	2008/01/18 09:36
L3	31129	l1 or l2	US-PGPUB; USPAT	OR	OFF	2008/01/18 09:37
L4	135	l3 adj (transform\$3 or tranfect\$3)	US-PGPUB; USPAT	OR	OFF	2008/01/18 09:37
L5	0	hybrid adj sensor adj kinase adj deficient	US-PGPUB; USPAT	OR	OFF	2008/01/18 09:37
L6	10	hybrid adj sensor adj kinase\$2	US-PGPUB; USPAT	OR	OFF	2008/01/18 09:38
L7	0	l4 same l6	US-PGPUB; USPAT	OR	OFF	2008/01/18 09:38
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L9	0	l4 same l8	US-PGPUB; USPAT	OR	OFF	2008/01/18 09:38
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L11	3	l4 and l10	US-PGPUB; USPAT	OR	OFF	2008/01/18 09:39

US 20050272924 A1 US-PGPUB
US 20040171695 A1 US-PGPUB
US 20040137594 A1 US-PGPUB
US 6803191 B2 USPAT
US 6768041 B2 USPAT
US 6716625 B1 USPAT
US 6673777 B1 USPAT
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US 6359198 B1 USPAT
US 6143728 A USPAT
US 5939306 A USPAT

US 20070185314 A1 US-PGPUB
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US 20060057607 A1 US-PGPUB
US 20040137594 A1 US-PGPUB
US 20030175930 A1 US-PGPUB
US 20030165932 A1 US-PGPUB
US 20030023032 A1 US-PGPUB
US 7208612 B2 USPAT
US 7183099 B2 USPAT

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1	US 20070248967 A1		US- PGPUB	20071025	101
2	US 20070134763 A1		US- PGPUB	20070614	77
3	US 20040137594 A1		US- PGPUB	20040715	37

	Title
1	Reporter Assay Using Secretory Luminescent Enzymes
2	Anti-virus therapy for respiratory diseases
3	Transformed cell with enhanced sensitivity to antifungal compound and use thereof